THE TOXICITY OF MONOVALENT AND DIVALENT CATIONS FOR SEA URCHIN EGGS.[1]

IRVINE H. PAGE.

The literature describing the action of salts on animal protoplasm (1–18), plant protoplasm (18–21), special physical systems (22–31) and the more specific case of the red blood cell seems to show that the toxicity of electrolytes is not of the same order for all types of cells. This fact renders it probable that protoplasm is not necessarily a "stuff" of uniform reactivity regardless of its origin.

"Salt toxicity" is probably the composite picture of many individual reactions. Salts have the most variant actions on lipoids, soaps, proteins, etc., and since protoplasm is made up of complex mixtures of these special moieties it is difficult to conceive in our present state of knowledge how we can ever predict "toxicity." This conception seems to have been overlooked in much of the investigative work concerning the toxicity of salts especially for highly differentiated organisms. Thus sodium might be very toxic for one tissue while nontoxic for another tissue and our term "toxicity," when applied to differentiated organisms, then becomes a miscellany of the greatest intricacy, on the one hand that of complex cellular reactivities and on the other complex cellular types.

Li and Na form one, and K, Rb, and Cs the other subdivision of the alkali metal period. If only the chemical structure and related physical properties of the reagent were to determine the physiological response of the protoplasm regardless of the protoplasmic type with which we deal we might anticipate that the relations which these metals maintain in the periodic system should be maintained in their action on protoplasm. The tendency should always be toward a grouping of Li and Na on the one hand and Rb, Cs, and K on the other. In addition Li and Mg should resemble Ca in some respects and Na in others.

[1] Contribution from the Research Division of Eli Lilly and Company, Marine Biological Laboratory, Woods Hole, Mass.

Many types of protoplasm respond to salts in such a way as to suggest that the specific chemical nature of the salt is, indeed, largely responsible for its reactions. However one must still appreciate the fact that the reagent electrolyte is only one half of the reacting system; the sum total of the effect we call "salt toxicity."

Although an explanation of toxicity is far from forthcoming nevertheless the data on toxicity are important in order to correlate other vistas of research on protoplasm with this complex phenomenon.

Methods.

The eggs of the Sea Urchin (*Arbacia punctulata*) were used in all the experiments. It may be stated at the outset that a possible source of error occurs in the resistance of individual eggs. However the difference observed in toxicity to the electrolytes are well beyond such experimental variations.

The eggs were removed directly from the ovaries with a pipette and placed in the isotonic solution of the purest electrolyte obtainable. Particular difficulty was experienced in obtaining a good quality $CaCl_2$. Freezing point determinations for Woods Hole sea water (G. Walden (32)) gave the following molar equivalents for the electrolytes. NaCl = 0.52, KCL = 0.53, $CaCl_2$ = 0.34, $MgCl_2$ = 0.35. The following are only approximate: Na_2HPO_4 =0.40, LiCl = 0.56, CsCl = 0.53, RbCl = 0.56.

Method 1.—In order to measure the effect of salts on protoplasm it is obvious that the salt must be in contact in a pure condition with the eggs. For instance, a mere trace of Ca or Mg profoundly modifies the toxicity of the monovalent cations. It is for this reason that it was found necessary to centrifuge the eggs three times in a solution of electrolyte to be tested adding fresh electrolyte at each centrifuging. The eggs were then transferred to Stender dishes, and 5 c.c. of the suspension removed at certain intervals of time, placed in a large quantity of sea water, washed and fertilized. After 24 hours the percentage development was estimated.

Curiously enough it is important that the eggs be taken directly from the ovaries particularly when the divalent cations are used (Heilbrunn (33)) and Page (32a). Eggs which have been washed

in sea water and allowed to stand, when placed in the electrolyte solution and centrifuged, quickly agglutinate. This has been shown by Chambers (34) to be due to the fact that $CaCl_2$ causes the jelly surrounding the egg to become very sticky after the eggs are returned to sea water whereas in KCl the jelly is simply dispersed. Due to the presence of $CaCl_2$ this adhesiveness causes the eggs to stick together so tightly that when centrifuged cytolysis occurs through mechanical tearing of the eggs. The same phenomenon occurs with NaCl but not in such a striking manner as with $CaCl_2$. Why fresh, unwashed eggs show much less tendency to clump on electrolyte treatment is difficult to say.

Method 2.[1]—Since it is possible that the relatively severe centrifugation employed in the first method might influence the results, we resorted to the following technique:

Eggs shed from the ovaries were very gently centrifuged in order to remove most of the sea water. Pyrex glass tubes 5 ft. long and ½ inch in diameter were filled with the electrolyte to be tested. The eggs were then mixed with a little of the salt solution and dropped into the top of the tube. As the eggs fell slowly through the solution a continuous washing process was in action. Convection and diffusion of the salts in the upper part of the tube into the lower part could hardly occur due to the length of the tubes and their relatively small bore. These washed eggs were then placed in large bowls of sea water, washed, and fertilized. At one and half and three hours, samples were removed and fixed in 3 drops of 20% formaldehyde to 3 c.c. of egg suspension. Counts were made in each sample, noting the number of eggs which were undivided and the number which were in each stage of division. By multiplying the numbers of two celled eggs by one, the four celled eggs by two, the eight celled by three, etc. and dividing the total number of divisions by the total number of eggs, the number of divisions per egg was determined. This figure was then corrected by the control (equalled 100%) which had been subjected to the same handling in sea waer. The temperature of the sea water varied between 20–23 degrees C. The $[H^+]$ of the electrolytes used as determined colorimetrically with the

[1] Our thanks are due Miss Kellicott for her work during this part of the investigation.

Clark and Lubs indicator series were Na Cl = 6.3, KCl = 6.4, $CaCl_2$ = 7.6, $MgCl_2$ = 6.3.

Results.

Figure 1 shows the results of treatment of Arbacia eggs with the various chlorides employing method 1 e.g. centrifuging the eggs with the electrolytes. The curves represent the average of ten

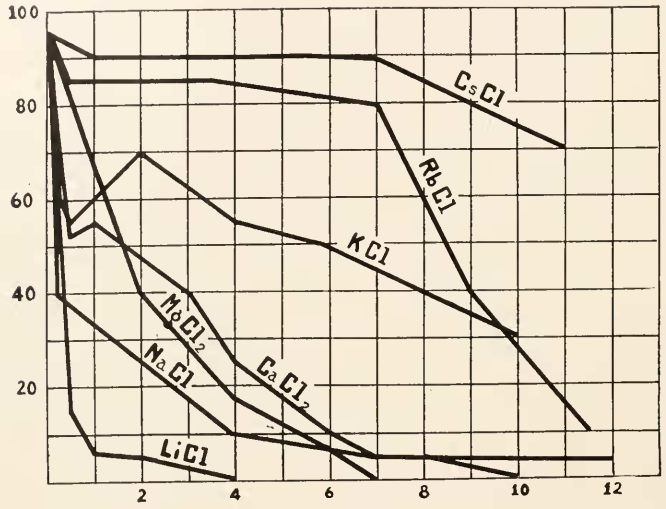

Fig. 1. Toxic effect of pure electrolytes on *Arbacia* eggs. The ordinate represents percentage of development after immersion for the period in hours in the electrolyte as represented by the abscissa. Method 1 in which centrifuging was employed to remove sea water.

experiments with each salt. The ordinates represent percentage development and the abscissæ the time of immersion in the salt solution before fertilization. It is evident from the chart that the cations can be arranged in the following toxicity series.

$$Li > Na > Mg, Ca > K > Rb > Cs.$$

Figures 2 and 3 show graphically the results of the average of nine experiments in which the divisions per egg corrected by the control is plotted against the time in the electrolyte. In Fig. 2 the eggs were allowed to develop for one and one-half hours after insemination and in Fig. 3 three hours, before being fixed in formalin and counted.

Potassium curiously enough exhibits a slight stimulation to division from two to three hours after treatment. Both methods

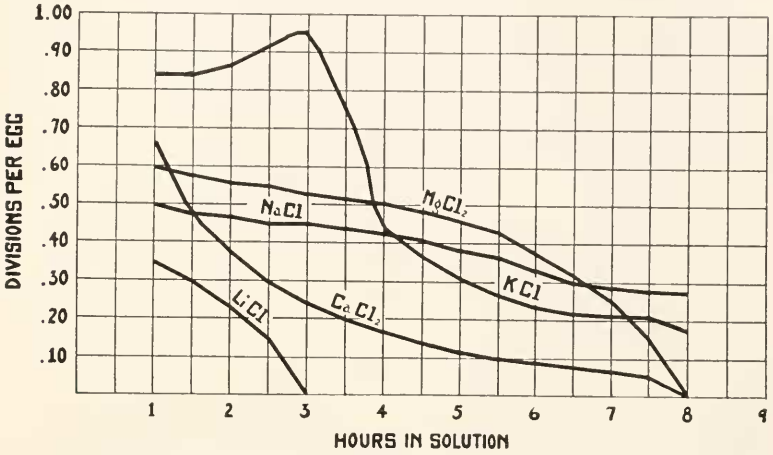

FIG. 2. The toxic effect of pure electrolytes on *Arbacia* eggs. The ordinates represent number of divisions per egg and the abscissæ represent hours in solution. Eggs were allowed to develop 1½ hours in sea water after fertilization. In Fig. 2 and Fig. 3 the number of divisions per egg has been corrected by the control.

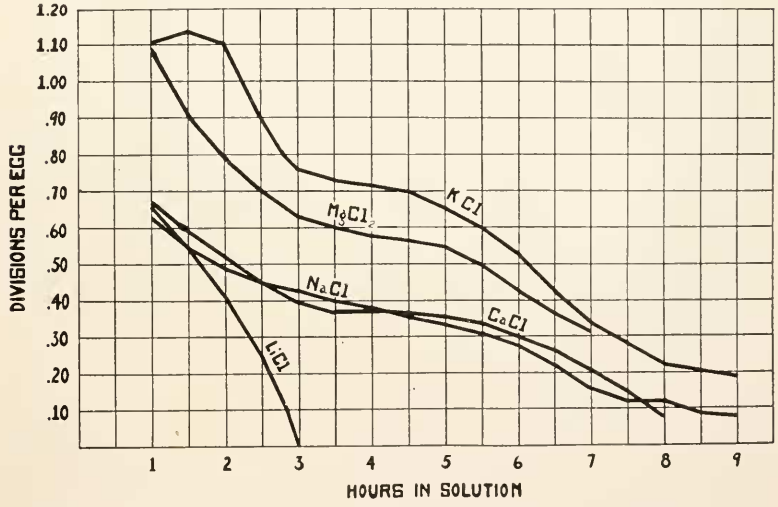

FIG. 3. Same as Fig. 2 except samples counted 3 hours after fertilization.

bring this out. $MgCl_2$ seems to be a little less toxic than $CaCl_2$ when centrifuging is not employed. We have the feeling that the toxicity of $CaCl_2$ depends somewhat on the quality of the salt used

The least toxic seems to be that of Poulenc (France). LiCl is uniformly the most toxic salt tested.

In order to show the effect of the presence of small amounts of sea water on the toxicity of the salt, eggs centrifuged three times adding fresh electrolyte solution after each centrifugation to rid them of sea water, were placed in varying mixtures of sea water and the electrolyte under investigation.

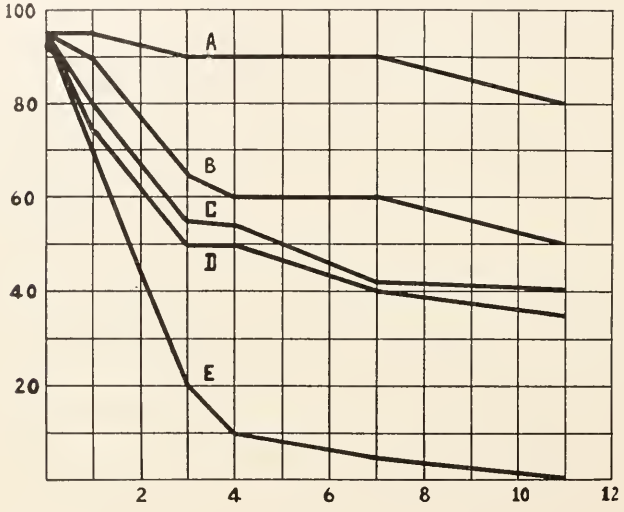

FIG. 4. Effect on toxicity of electrolytes when varying amounts of sea water are present. Ordinates represent percentage development after immersion in the electrolyte solution and abscissæ the numbers of hours of treatment. Curve *A* represents a mixture of 10 parts $CaCl_2$ plus 30 parts sea water; Curve *B* = 22 parts $CaCl_2$ plus 18 parts sea water; Curve C = 28 parts $CaCl_2$ plus 12 parts sea water; curve *D* = eggs centrifuged once from surrounding sea water and put in isotonic $CaCl_2$; curve *E* = eggs centrifuged three times with pure $CaCl_2$ to remove all trace of sea water.

The following figures (4 and 5) illustrate these results with Ca, Na and K. The other salts show similar effects. As in Fig. 1 the ordinates represent percentage of eggs which fertilized developed into the swimming stage and the abscissæ the time of treatment with the electrolyte before fertilization.

The smallest amount of sea water present reduces the salt toxicity in a most impressive manner. It makes one realize what a potent agent ionic antagonism may be.

It seems perfectly clear that the toxicity of the electrolytes as measured by the ability of the egg to be fertilized and subsequently develop, determined by two separate methods and two observers, follows the series Li > Na > Ca, Mg > K > Rb > Cs. What is not clear is the interpretation of these results.

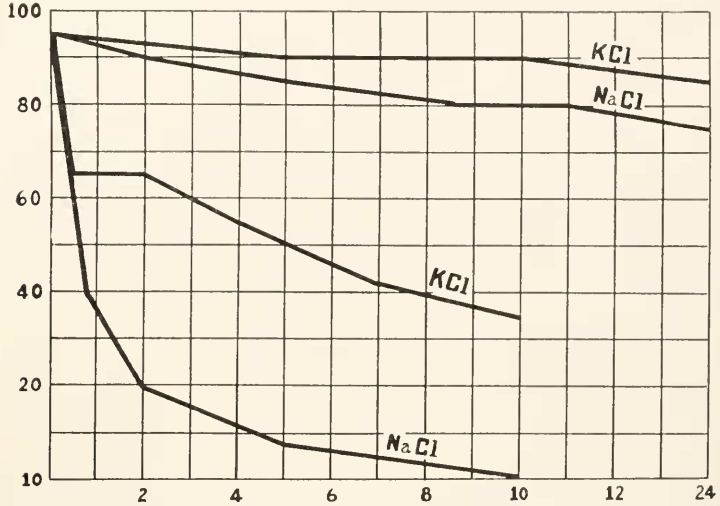

FIG. 5. Effect on toxicity of NaCl and of KCl of varying amounts of sea water Ordinates represent percentage development after immersion for the period in hours represented by the abscissæ. The two upper curves show the toxicity when the eggs are centrifuged once and placed in the isotonic salt. The two lower curves represent the toxicity when the eggs are centrifuged three times with the electrolyte.

It is suggestive that the atomic numbers, show a series very nearly identical with the order of toxicity found in this study. In order of increasing atomic number the series runs Li, Na, Mg, K, Ca, Rb, Cs.

However it is probably fortuitous that the toxicity series (with the exception of K) found for the Sea Urchin egg closely parallels the atomic number series. Any proof to the contrary must consider the action of the salts on at least the more important protein, lipoid, fat and mineral systems which constitute protoplasm and the complex interrelations of these various systems. It is possible however that this parallelism may represent the dominance of the chemical constitution of the reagent in the reaction between protoplasm and the salt.

The problem is further complicated by the fact that Chambers and Reznikoff (35, 36) have shown that the toxicity of the salts for fresh water Amœbæ immersed in the solution is

$$K > Na > Ca > Mg,$$

but when the electrolyte is injected into the interior of the protoplasm quite the reverse relation holds, viz.,

$$Ca > Mg > Na > K.$$

These results seem to implicate the chemically active plasma membrane at the surface of the cell and possibly the difference in type of protoplasm ("interior" and "exterior") as suggested by Chambers (37) and Page, Chambers and Clowes (38).

Summary.

1. The toxicity of the following cations used as chlorides for the Arbacia egg was found to be

$$Li > Na > Ca, \quad Mg > K > Rb > Cs.$$

2. It has been suggested that the term "toxicity" is applied to a miscellany of the most heterogeneous reactants in protoplasm hence as an entity its prediction and formulation in our present state of knowledge is not probable.

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